

**Seasonal variations in concentration and lability of dissolved
organic carbon in Tokyo Bay**

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Abstract

Concentrations of recalcitrant and bioavailable dissolved organic carbon (DOC) and their seasonal variations were investigated at three stations in Tokyo Bay, Japan, and in two freshwater sources flowing into the bay. On average, recalcitrant DOC (RDOC), as a remnant of DOC after 150 days of bottle incubation, accounted for 78% of the total DOC in Shibaura sewage treatment plant (STP) effluent, 67% in the upper Arakawa River water, 66% in the lower Arakawa River water, and 78% in surface bay water. Bioavailable DOC (BDOC) concentrations, defined as DOC minus RDOC, were lower than RDOC at all stations. In freshwater environments, RDOC concentrations were almost constant throughout the year. In the bay, RDOC was higher during spring and summer than autumn and winter because of freshwater input and biological production. The relative concentration of RDOC in the bay derived from phytoplankton, terrestrial, and open oceanic waters was estimated to be 8–10%, 21–32%, and 59–69%, respectively, based on multiple regression analysis of RDOC, salinity, and chl *a*. In addition, comparison with previous data from 1972 revealed that concentrations of RDOC and BDOC have decreased by 33% and 74% at freshwater sites and 39% and 76% at Tokyo Bay, while the ratio of RDOC to DOC has increased. The change in DOC concentration and composition was probably due to increased amounts of STP effluent entering the system. Tokyo Bay exported mostly RDOC to the open ocean because of remineralization of BDOC.

1. Introduction

The dissolved organic carbon (DOC) pool is the largest organic carbon reservoir in the ocean and contains 662 Pg of carbon, which is roughly equivalent to that stored in the atmosphere in the form of carbon dioxide (Hansell et al., 2009). In open oceans, DOC production is ultimately constrained by primary production (e.g., Carlson, 2002). In coastal waters, DOC consists of diverse mixtures of carbon with varying timescales of lability formed by primary production and materials of terrestrial origin. Riverine DOC export to the open ocean has been estimated to range from 0.21 to 0.25 PgC yr⁻¹ (Meybeck, 1993; Ludwig et al., 1996; Hedges et al., 1997; Cauwet, 2002) without considering loss or gain of DOC in coastal waters. Coastal waters are typically considered passive conduits in regional and global carbon budgets (Cole et al., 2007; Aufdenkampe et al., 2011; Regnier et al., 2013). However, degradation of terrestrial DOC and biological production of DOC in coastal regions can significantly modify the flux of DOC to the open ocean. Dai et al. (2012) recently reported that riverine DOC export to the open ocean would be reduced to 0.17 PgC yr⁻¹ if 10% was degraded in coastal waters. However, their assumption of 10% was based on the results of only a few bottle incubation experiments (Amon and Benner, 1996; Raymond and Bauer, 2000; Moran et al., 1999). Therefore, to better understand DOC export to the open ocean, experimental data describing DOC lability, preferably from different environmental locations and different seasons, are needed.

In this study, we measured seasonal variations in the concentration and lability of DOC in Tokyo Bay, Japan, to evaluate the significance of DOC degradation to the carbon budget in coastal waters and carbon export to the open ocean. The bay is semi-enclosed, with an area of about 922 km² and a mean water depth of 19 m. The residence

time of water in the bay is estimated to be about 50 days (Takada et al., 1992). The bay is located in central Japan and surrounded by metropolitan areas, with a total population of about 26 million. Tokyo Bay represents typical highly urbanized coastal waters, which are rapidly expanding worldwide (Nellemann et al., 2008). We also compared our results with those obtained by Ogura (1975), who carried out an investigation of Tokyo Bay in the 1970s and found that DOC in coastal waters could be divided into bioavailable DOC (BDOC) and recalcitrant DOC (RDOC). Owing to his investigation, BDOC and RDOC data from 1972 are available for Tokyo Bay.

2. Materials and Methods

Freshwater samples were collected two and eight times from the upper and lower Arakawa River, respectively, and five times from effluent of the Shibaura sewage treatment plant (STP; Figure 1) between December 2011 and October 2013. Freshwater samples were collected using a bucket, transferred into HCl acid-washed 1-L polyethylene bottles and kept in the dark until being processed in the laboratory. The bucket and sample bottles were rinsed three times with sample water before being filled. Within 2 h of after sample collection, the freshwater samples were carried back to the laboratory. DOC and the degradation experiment samples were filtered immediately after arrival in the laboratory through GF/F filters (nominal pore size; 0.7 μm) that had been precombusted at 450°C for 3 h. Surface seawater of Tokyo Bay was collected in 8-L Niskin bottles mounted on a CTD rosette on the R/V Seiyō-maru of Tokyo University of Marine Science and Technology from January 2012 to December 2012 monthly at three stations (Figure 1). Within 1 h after sample collection, DOC and the degradation experiment samples were filtered through precombusted GF/F filters on board. Then,

81 samples were kept in the dark and carried back to the laboratory within 4 h. We
82 assumed that GF/F filters allow the passage of a significant fraction of free-living
83 bacteria into DOC samples (e.g. Bauer and Bianchi, 2011). In addition, Tranvik and
84 Höfle (1987) investigated the interactions between bacterial assemblages and DOC
85 consumption using batch cultures and found that the DOC bioavailability was
86 independent of the inoculum. Tanaka et al. (2011) also showed that mineralization rate
87 of the BDOC fraction in coral reef was not different from natural waters and waters
88 filtrated by GF/F, nevertheless the initial bacterial abundance in the incubated waters
89 filtrated by GF/F was about 30-50% of bacteria abundance in natural waters. Therefore,
90 we did not add the microbial community. We also did not add nutrients for the
91 degradation experiment because we assumed nutrients were not limiting the microbial
92 growth (see section 3.1.). Degradation experiment samples were then transferred to 600-
93 mL amber glass bottles and stored at room temperature (20°C) in total darkness until
94 analysis. The 100 mL headspace in each glass bottle contains about 800 μmol oxygen.
95 The highest initial DOC concentration in this study was 430 $\mu\text{mol L}^{-1}$ (Table 1). If we
96 assume that one mole of oxygen is consumed when one mole of organic carbon is
97 mineralized into CO_2 , oxygen in headspace should have provided sufficient oxygen
98 supply for heterotrophic decomposition by bacteria. The degradation experiments were
99 conducted based on a total of seven incubations (0, 5, 10, 20, 50, 100, and 150 days) per
100 field sampling event. After incubation, samples were dispensed into glass vials that had
101 been pre-washed with HCl, pure water (Milli-Q water, Millipore Corp., Bedford, MA,
102 USA), and then pre-combusted. Freshwater samples were preserved with 6 mol L^{-1} HCl
103 at a concentration corresponding to 1% of the sample volume, then stored in a
104 refrigerator (5°C). Tokyo Bay samples were frozen (-25°C) without adding HCl. DOC

samples were measured at least in triplicate with a TOC analyzer (TOC-V_{CSH}, Shimadzu, Kyoto, Japan). Potassium hydrogen phthalate (Wako Pure Industries, Osaka, Japan) was used as a standard for measurement of DOC. DOC blank including pure water, instrument blank, and any carbon derived from vial was about 3 $\mu\text{mol L}^{-1}$ in total.

RDOC was here defined as the concentration of DOC remaining at 150 days and BDOC was obtained by subtracting RDOC from the initial DOC (Lønborg et al., 2009). The degradation rate of DOC was described by a first-order exponential decay model with a constant RDOC pool:

$$\text{DOC}(t) = \text{BDOC} \cdot \exp(-k \cdot t) + \text{RDOC} \quad (1)$$

where $\text{DOC}(t)$ is the amount of DOC remaining at time t (day), k is the degradation rate constant (day^{-1}), and RDOC is the remaining DOC pool after 150 days of incubation. BDOC is the bioavailable DOC ($\mu\text{mol L}^{-1}$) at the beginning of incubation and practically equals to subtraction of RDOC from initial DOC. Using BDOC and RDOC concentrations, k can be estimated by fitting the observed $\text{DOC}(t)$ values to equation (1) using Matlab 2012a. For comparison with the results reported by Lønborg and Álvarez-Salgado (2012), we used the following equation to normalize the degradation rate to the rate at 15°C,

$$k(15^\circ\text{C}) = k(T) \cdot (Q_{10})^{\frac{T-15}{10}} \quad (2)$$

where $k(15^\circ\text{C})$ and $k(T)$ are the degradation rate constants at 15°C and $T^\circ\text{C}$ (20°C for our experiment). Q_{10} is the temperature coefficient. In this study, we used a value of 2.2

based on Lønborg and Álvarez-Salgado (2012).

Temperature and salinity were measured in the field using a YSI EC 300 (YSI/Nanotech Inc., Yellow Springs, OH, USA) at freshwater sites and a CTD (Falmouth Scientific Inc., Bourne, MA, USA) for sites in the bay. Water samples for chlorophyll *a* (chl *a*) measurement were filtered through precombusted (450°C, 3h) GF/F filters. After filtration, chlorophyllous pigments were extracted using N, N-dimethylformamide, and the concentrations of chl *a* were determined by the fluorometric method (Suzuki and Ishimaru, 1990) using a fluorometer (TD-700, Turner Designs, Sunnyvale, CA, USA). Samples for particulate organic carbon (POC) were filtered through precombusted (450°C, 3h) GF/F filters, after which the filters were stored at –80°C until analysis. The samples for POC analyses were dried at 60°C and acidified with vapor at 12 mol L⁻¹ HCl to remove carbonate before analysis. POC were measured using a Hydra 20-20 isotope ratio mass spectrometer coupled to an ANCA-GSL elemental analyzer (SerCon Ltd., Crewe, UK).

3. Results and Discussion

3.1. Nutrient Conditions in Tokyo Bay

Nutrient concentrations in freshwater and Tokyo Bay sites were high throughout the year (Table S1 and S2). During summer, the phosphorus concentration generally decreased and the nitrogen/phosphorus ratio was higher than the Redfield ratio of 16 (Redfield et al., 1963), suggesting that phosphorus acts as a limiting factor of primary production at the bay. A degradation experiment with phosphate (KH₂PO₄, 2 µmol L⁻¹) was conducted to ensure that phosphorus was not a limiting factor in July 2012, at which time the concentration of phosphate was lowest in the year (0.1 µmol L⁻¹; Table

S1 and S2). The results of the degradation experiment with added phosphorus were not significantly different from those of the degradation experiment without added phosphorus ($y=1.1x-8.2$, $R^2=0.97$, $p<0.05$). We did not add nutrients for the degradation experiment because we assumed nutrients were not limiting the microbial growth.

3.2. Lability and sources of freshwater DOC flowing into Tokyo Bay

The lowest chl *a*, DOC, and POC concentrations were observed at the upper Arakawa River station, which is considered to be pristine (Table 1). The average concentration of DOC was $38 \mu\text{mol L}^{-1}$ at the upper Arakawa River station. Headstream water sources in Japan are mostly surface runoff from neighboring watersheds and ground water input through the mineral soil horizon before entering surface water (Nakamura et al., 2011). The precipitation is characterized by very low DOC concentrations (Avery et al., 2003). Ground water inputs through the mineral soil horizon typically have low DOC concentrations because mineral soils have the ability to adsorb a significant amount of DOC (Aitkenhead et al., 2003). Such low concentrations of DOC in headstream waters have commonly been reported in Japan (e.g. Maki et al., 2010), as well as in other countries (e.g. Yamashita et al., 2011). The results of the DOC degradation experiments at the upper Arakawa River station are shown in Figure 2(a). Rapid degradation of the labile pool was observed within the first 20 days of incubation. Additionally, the average concentration of RDOC was $25 \mu\text{mol L}^{-1}$, which was the lowest value in freshwater and Tokyo Bay sites and its contribution to the total DOC was 67%.

Relatively high temperatures and DOC values were observed at Shibaura STP, while seasonal variations in chl *a* and POC were relatively small (Table 1). The average

concentration of DOC was $355 \mu\text{mol L}^{-1}$, which was about nine times higher than the value at the upper Arakawa River station. The annual mean concentration of RDOC was $278 \mu\text{mol L}^{-1}$, while the mean contribution of RDOC to the total DOC was 78% (Figure 2(b)). The RDOC concentrations did not vary greatly between observation months, and a significant linear relationship was observed between BDOC and DOC ($R^2=0.976$, $p<0.001$, slope=1.16), indicating that the seasonal variations in DOC were mostly due to variations in the bioavailable fraction. Typically, STP effluents have high organic carbon concentrations and a large bioavailable fraction (Servais et al., 1995; Servais et al., 1999; Kaushal and Belt, 2012). In contrast, effluent of Shibaura STP showed a high proportion of RDOC (67–93%). These findings suggest that most of the BDOC were degraded before being discharged. This likely occurred because STPs in Japan conduct secondary treatment, which consists of removal of wastewater suspended solids by sedimentation and degradation of dissolved organic matter by activated sludge treatment (Kadlec and Wallace, 2008).

Relatively high chl *a* and POC concentrations were observed at the lower Arakawa River station (Table 1). The maximum concentrations of chl *a*, DOC, and POC were observed in spring. The average concentration of DOC was $235 \mu\text{mol L}^{-1}$, while the annual mean concentration of RDOC was $149 \mu\text{mol L}^{-1}$ and the mean contribution of RDOC to the total DOC was 66% (Figure 2(c)). The concentrations of DOC were more than six times higher than those at the upper Arakawa River station. High concentrations of nutrients were also observed at the lower Arakawa River station (see Table S1 and S2 in the auxiliary material), which was likely a result of inputs of DOC and nutrients from STPs between observation sites. The RDOC concentrations did not show large differences between observation months, and a significant linear relationship

between BDOC and DOC was observed ($R^2=0.942$, $p<0.001$, slope=1.12), indicating that the seasonal variations of DOC at the lower Arakawa River station were due to variations in the bioavailable fraction.

Freshwater flowing into Tokyo Bay primarily consists of a mixture of river water and STP effluent. The total discharge ratio of river water to STP effluent in the bay is about 1:1 (Japan Sewage Works Association, 2010; Bureau of Sewerage, 2013). Assuming that the ratio of river water to STP effluent is 1:1 and that data collected at the upper Arakawa River station and Shibaura STP represent these two sources, the average concentrations of RDOC and BDOC in freshwater would be 152 and 47 $\mu\text{mol L}^{-1}$, respectively. These values are comparable with those observed at the lower Arakawa River station (149 and 86 $\mu\text{mol L}^{-1}$, respectively). Arakawa River, which is the largest river flowing into the bay, accounts for about 30% of the freshwater discharge (Nihei et al., 2007a). Most rivers flowing into the bay have similar water quality because of similar land use within the drainage basin (Nihei et al., 2007b); accordingly, we can reasonably assume that observed RDOC and BDOC concentrations at the lower Arakawa River station represent concentrations of total river water flowing into Tokyo Bay.

Table 2 summarizes the first-order decay constants obtained by fitting the exponential degradation of DOC with time. The annual average degradation rate constant normalized to 15°C at the lower Arakawa River station was $0.031\pm0.005\text{ d}^{-1}$, which was similar to other coastal waters ($0.066\pm0.065\text{ d}^{-1}$; Lønborg and Álvarez-Salgado, 2012).

3.3. Tokyo Bay

Seasonal variations in temperature, salinity, chl *a*, POC, and DOC at the three stations in Tokyo Bay are presented in Figure 3. High values of temperature, chl *a*, POC, and DOC were observed during spring and summer, while low values were observed during autumn and winter. Salinity was higher during autumn and winter than spring and summer. DOC concentrations ranged from 81 to 182, 76 to 153, and 60 to 108 $\mu\text{mol L}^{-1}$ at stations F3, F6, and 06, respectively (Figure 3). The concentrations of DOC were generally lower than these at the lower Arakawa River station.

3.3.1. Lability of DOC

Rapid degradation of the labile pool occurred within the first 20 days of incubation, indicating that BDOC were remineralized during the residence time of the bay water (Figure 4). The seasonal variations in DOC, RDOC, and BDOC concentrations at the three stations in Tokyo Bay are shown in Figure 5. RDOC ranged from 70 to 120 $\mu\text{mol L}^{-1}$ at F3, 58 to 130 $\mu\text{mol L}^{-1}$ at F6, and 48 to 80 $\mu\text{mol L}^{-1}$ at 06. The mean contributions of RDOC to the total DOC were 81% at F3, 77% at F6 and 72% at 06. Both RDOC and BDOC showed similar seasonal variations as DOC, with high variations being observed in spring and summer and low in autumn and winter. The contribution of RDOC to the total DOC was higher than that of BDOC at all stations for the entire observation period. The RDOC concentrations of the surface water were significantly higher than those of the bottom water at 06 (see Table S3 in the auxiliary material). Thus, our RDOC results likely include a fraction of semi-labile DOC. Degradation of this semi-labile DOC fraction would occur by bacterial mineralization with longer time, photo-degradation (Moran and Zepp, 1997; Opsahl and Benner, 1997; Obernosterer and Benner, 2004), aggregation (Sholkovitz, 1976; Mulholland, 1981), and/or sorption to

particles (Chin et al., 1998; Kerner et al., 2003). However, the results of this study did not change significantly when DOC were divided into BDOC, semi-labile DOC, and RDOC. The lifetime of semi-labile DOC is about 1.5 years (Hansell, 2013), which is considerably longer than the residence time of Tokyo Bay (Takada et al., 1992). Therefore, in our analysis, there was no problem with inclusion of semi-labile DOC in RDOC. In addition, Ogura (1975) only divided DOC into BDOC and RDOC; therefore, we divided DOC in the same way to enable comparison with that study.

Table 3 summarizes the degradation constants of DOC for the bay surface waters. The annual average degradation rate constants normalized to 15°C at F3, F6, and 06 were 0.128 ± 0.014 , 0.094 ± 0.016 , and $0.083 \pm 0.010 \text{ d}^{-1}$, respectively. Most degradation rate constants for the bay water were higher than those of freshwater (Tables 2 and 4). The half-lives of BDOC were calculated from the degradation rate constant. The annual average half-lives of BDOC at F3, F6, and 06 were 5.4, 7.4, and 8.4 days, respectively. BDOC produced by phytoplankton in the bay water might have led to faster degradation rates because the half-lives of BDOC were about five times faster than the residence time of the bay water.

RDOC concentrations in Tokyo Bay were negatively correlated with salinity and positively correlated with chl *a* (Table 4). In the bay, salinity was lower in spring and summer than in autumn and winter (Figure 3) because of high freshwater input during spring and summer. The freshwater RDOC concentration was higher than that of Tokyo Bay water; therefore, a negative relationship between RDOC and salinity was observed. RDOC is also produced directly by phytoplankton (Kragh and Søndergaard, 2009). Hence, the positive relationship between RDOC and chl *a* observed in this study likely reflected RDOC produced by phytoplankton.

3.3.2. RDOC sources

To estimate the sources of RDOC in Tokyo Bay, multiple linear regression analysis with salinity and chl *a* as the independent variables was applied to all RDOC data observed at three stations in Tokyo Bay. BDOC in Tokyo Bay was not well correlated with salinity and chl *a* (Table 4), so multiple linear regression analysis was not applied to the BDOC data. We obtained the following multiple linear regression equation (Model I):

$$[\text{RDOC}] = (259 \pm 38) - (5.96 \pm 1.20) \times [\text{Sal}] + (0.597 \pm 0.20) \times [\text{Chla}] \quad (3)$$

$(r^2 = 0.79, P < 0.001, n = 35)$

where [RDOC] is the RDOC concentration ($\mu\text{mol L}^{-1}$), [Sal] is salinity, and [Chla] is the chlorophyll *a* concentration ($\mu\text{g L}^{-1}$) of each sample. The end-member of terrestrial RDOC ($[\text{RDOC}_{\text{terr-end}}]$) was as follows when the salinity was 0;

$$[\text{RDOC}_{\text{terr-end}}] = (259 \pm 38) + (0.597 \pm 0.20) \times [\text{Chla}_{\text{river}}] \quad (4)$$

where $[\text{Chla}_{\text{river}}]$ is the chl *a* concentration ($\mu\text{g L}^{-1}$) at the freshwater site. The end-member of terrestrial RDOC was higher than the average RDOC concentration at the lower Arakawa River station ($149 \mu\text{mol L}^{-1}$) and was similar to that of Shibaura STP ($278 \mu\text{mol L}^{-1}$). The ratio of river water to STP effluent was 1:1 (Japan Sewage Works Association, 2010; Bureau of Sewerage, 2013) and data collected at the upper Arakawa River station and Shibaura STP represent these two sources (see section 3.1.). It is

possible that freshwater inputs in Tokyo Bay were more strongly influenced by STPs than headstream waters. Alternatively, if we assume that the RDOC concentration at salinity=0 and chl *a*=0 was close to the average RDOC concentration actually observed at the lower Arakawa River station (149 $\mu\text{mol L}^{-1}$), we obtain the following multiple regression equation (Model II):

$$[\text{RDOC}] = 149 - (2.65 \pm 0.26) \times [\text{Sal}] + (1.03 \pm 0.40) \times [\text{Chla}] \quad (5)$$

$(r^2 = 0.71, P < 0.001, n = 35)$

The end-member of terrestrial RDOC ($[\text{RDOC}_{\text{terr-end}}]$) is as follows when salinity is 0;

$$[\text{RDOC}_{\text{terr-end}}] = 149 + (1.03 \pm 0.40) \times [\text{Chla}_{\text{river}}] \quad (6)$$

In this study, we assumed that $[\text{Chla}_{\text{river}}]$ was $6.0 \mu\text{g L}^{-1}$ (Ministry of the Environment: <http://www.env.go.jp>), which was the average value of surface waters in Arakawa River. Although $[\text{Chla}_{\text{river}}]$ is usually lower than $10 \mu\text{g L}^{-1}$ throughout the year, phytoplankton blooms occasionally persist (Ministry of the Environment: <http://www.env.go.jp>). Calculation of the RDOC sources using the minimum and maximum chl *a* concentration at the lower Arakawa River station (Table 1) resulted in estimated RDOC sources that did not differ significantly from the minimum and maximum concentrations.

The concentrations of RDOC in the open ocean ($[\text{RDOC}_{\text{ocean-end}}]$) can be estimated by assuming that salinity and chl *a* in the open ocean were 34.5 (Okada et al., 2007) and $1.0 \mu\text{g L}^{-1}$, respectively (Japan Meteorological Agency: <http://www.jma.go.jp/jma/index.html>), which were the average values of surface waters

offshore from Tokyo Bay. The $[RDOC_{ocean-end}]$ values were 54.0 ± 3.2 and 58.6 ± 8.6 $\mu\text{mol L}^{-1}$ for Model I and II, respectively, which were comparable to the annual average RDOC concentration of the bottom water at 06 (see Table S3 in the auxiliary material). Following the method described Ogawa and Ogura (1990), we estimated the contributions of RDOC from different sources (RDOC from the open ocean $[RDOC_{ocean origin}]$, terrestrial RDOC $[RDOC_{terr}]$, and RDOC from phytoplankton $[RDOC_{phyto}]$) using two models of the multiple linear regression analysis. The RDOC concentrations can be expressed as follows:

$$[RDOC] = [RDOC_{phyto}] + [RDOC_{ocean origin}] + [RDOC_{terr}] \quad (7)$$

The equation describing RDOC derived from the open ocean ($[RDOC_{ocean origin}]$) is as follows:

$$[RDOC_{ocean origin}] = [RDOC_{ocean-end}] \times [Sal]/34.5 \quad (8)$$

The terrestrial RDOC ($[RDOC_{terr}]$) is as follows:

$$[RDOC_{terr}] = [RDOC_{terr-end}] \times (34.5 - [Sal])/34.5 \quad (9)$$

The RDOC derived from phytoplankton ($[RDOC_{phyto}]$) can be estimated from equation (7):

$$[RDOC_{phyto}] = [RDOC] - [RDOC_{ocean origin}] - [RDOC_{terr}] \quad (10)$$

343

344 For each multiple linear regression equation (equation 3-6), the two sided 95%
345 confidence bounds of each coefficient and intercept were estimated. For the
346 concentrations of RDOC originating from phytoplankton, terrestrial, and open oceanic
347 waters, we estimated the upper and lower bounds by changing an equation within its
348 error range. The relative concentrations of RDOC (%) with error in the bay originating
349 from phytoplankton, terrestrial, and open oceanic waters at the three stations are
350 presented in Table 5. The results show that the open ocean is the major source of RDOC
351 in Tokyo Bay. At station F3, which is located close to land, terrestrial RDOC was
352 comparable to that from the open ocean. The concentration of terrestrial RDOC was
353 significantly higher than that of RDOC from phytoplankton at all stations, even at the
354 bay mouth.

355 The influx of terrestrial TOC (POC+DOC) from the rivers to Tokyo Bay was
356 estimated using a mass balance model (8.1×10^{10} gC year⁻¹; Yanagi et al., 1993), and the
357 DOC/TOC ratio in freshwater site was 0.62 (Kubo, unpublished data). Hence, the influx
358 of terrestrial DOC was estimated to be 5.0×10^{10} gC year⁻¹ and RDOC accounted for
359 66% of terrestrial DOC (see section 3.2.; 3.3×10^{10} gC year⁻¹). The efflux of TOC from
360 the surface bay to the open ocean was estimated using a mass balance model (9.4×10^{10}
361 gC year⁻¹; Yanagi et al., 1993), and the DOC/TOC ratio in the surface bay mouth was
362 0.69 (Kubo, unpublished data). Hence, the efflux of DOC was estimated to be 6.5×10^{10}
363 gC year⁻¹ and RDOC accounted for 73% in the surface bay mouth (see section 3.3.;
364 4.7×10^{10} gC year⁻¹). Assuming that terrestrial and phytoplankton RDOC were exported
365 outside of the bay in the same ratio at the bay mouth (Table 5), Tokyo Bay exported
366 mostly terrestrial RDOC to the open ocean owing to the high concentration of terrestrial

RDOC and remineralization of BDOC. Moreover, the ratio of terrestrial RDOC input into the bay (3.3×10^{10} gC year⁻¹) and terrestrial RDOC efflux to the open ocean (0.9×10^{10} and 0.6×10^{10} gC year⁻¹, respectively for Model I and II) was 28% and 17%, respectively. Residual terrestrial RDOC in the bay may be removed from the water column by photo-degradation (Moran and Zepp, 1997; Opsahl and Benner, 1997; Obernosterer and Benner, 2004), aggregation (Sholkovitz, 1976; Mulholland, 1981), and/or sorption to particles (Chin et al., 1998; Kerner et al., 2003).

The fate of terrestrial DOC in the coastal ocean and the open ocean has long been the subject of debate (Hedges et al., 1997). For example, biomarkers (e.g. lignin phenols) and the stable carbon isotopic composition of DOC are commonly used to estimate the contribution of terrestrial DOC to the open ocean (Druffel et al., 1992; Hedges et al., 1997; Raymond and Bauer, 2001; Bauer and Bianchi, 2011). Lignin phenols analysis indicated that terrestrial DOC comprises only a small fraction (4–10%) of the total DOC in the open ocean (Meyers-Schulte and Hedges, 1986; Opsahl and Benner, 1997; Hernes and Benner, 2006). In addition, the stable carbon isotopic composition of DOC also indicated that terrestrial DOC represents less than 10% of the total DOC (Bauer et al., 2002). As a result, most terrestrial DOC is remineralized in coastal waters, and only a small fraction is exported to the open ocean. In this study, terrestrial RDOC in the surface bay mouth accounted for less than 20% of the total RDOC (Table 5). Although these levels were slightly higher than those reported in previous studies using lignin phenols and stable carbon isotopic compositions of DOC, they are probably reasonable given that exported terrestrial RDOC were further diluted with open oceanic water once outside the bay. Nevertheless, more complete information regarding the sources and lability of DOC are important to enable a better understanding

of the fate of DOC in the coastal ocean and open ocean.

3.4. Change of DOC over four decades

Ogura (1975) investigated the concentrations of RDOC and BDOC in Tokyo Bay and freshwater sources flowing into the bay in the 1970s using GF/C filters (nominal pore size; 1.2 μm) to collect filtrate of degradation samples and found that the contribution of the DOC fraction from 0.45 μm (Millipore HA filter, Millipore Corp., Bedford, MA) to 1.2 μm was about 10% of the total DOC in Tokyo Bay. Ogawa and Ogura (1992) also showed that the low molecular weight DOC ($< 10,000$ Dalton; < 0.2 μm) in the bay comprised a major portion of the total DOC filtered by 1.2 μm (78–97%). Hence, the DOC fraction from 0.7 μm to 1.2 μm comprised a minor proportion of the DOC in Tokyo Bay. Ogura (1975) used a wet chemical oxidation method to measure the samples, while Ogawa and Ogura (1992) showed that both a wet chemical oxidation method and high temperature catalytic oxidation method for measuring DOC concentrations of Tokyo Bay waters generated similar results. Ogura (1975) conducted degradation experiment with short incubation time (about 50 days) relative to our experiment (150 days). However, BDOC can be consumed by bacteria over short periods (days to weeks; Lønborg and Álvarez-Salgado, 2012) and therefore, the remaining DOC pool after 150 days of incubation, used in equation (1), is not significantly different from RDOC concentration at 50 days. Actually, degradation rate constants calculated using data from the first 50 days (k_{50}) and those using all data from 150 days of experiment (k_{150}) are not significantly different ($k_{50}=0.90\times k_{150}+0.016$, $R^2=0.86$, $p<0.01$). Therefore, we assume that our degradation rate constants are comparable to those reported by Ogura (1975).

In 1972, the average concentrations of RDOC and BDOC were 224 and 337 $\mu\text{mol L}^{-1}$ (40% and 60% of the total DOC, respectively) in the freshwater environment of the lower Tamagawa River, which flows into Tokyo Bay (Ogura, 1975). The present RDOC and BDOC concentrations at the lower Arakawa River station (149 and 86 $\mu\text{mol L}^{-1}$) are lower than those reported by Ogura (1975). If we assumed that the amount of freshwater discharge into the bay has increased by 24% (Okada et al., 2007), the amount of RDOC and BDOC flowing into the bay would have decreased by 17% and 68%, respectively. Ogura (1975) also estimated a degradation rate constant (k_{15}) of 0.087 d^{-1} , which is much higher than that observed in the present study (Table 2). These changes are consistent with the fact that proportion of treated wastewater to the total freshwater inflow to the bay increased from 11% to 28% from 1970 to 2000 (National Institute for Land and Infrastructure Management, 2004). Degradation of DOC at STPs before being discharged should lower BDOC fraction more than RDOC. Overall, our results indicate that the quantity of DOC flowing into the bay has decreased, and the quality of DOC becomes more recalcitrant.

In Tokyo Bay, the concentrations of DOC at station F3 decreased from 287 $\mu\text{mol L}^{-1}$ in 1972 (Ogura, 1975) to 124 $\mu\text{mol L}^{-1}$ in 2012, most likely because of a decrease of DOC discharge from rivers and a decrease in primary production (Yamaguchi and Shibata, 1979; Yamaguchi et al., 1991; Bouman et al., 2010). The concentrations of RDOC and BDOC observed in this study (100 $\mu\text{mol L}^{-1}$ and 24 $\mu\text{mol L}^{-1}$, respectively) were lower than those estimated by Ogura (1975) in 1972 (165 $\mu\text{mol L}^{-1}$ and 100 $\mu\text{mol L}^{-1}$, respectively). Conversely, the contribution of RDOC to the total DOC in this study (80.6%) is higher than the value observed in 1972 (57.5%; Ogura, 1975). The concentrations of RDOC and BDOC in Tokyo Bay have decreased because of a

decrease in DOC load from the land, especially for BDOC. As a result, DOC becomes more recalcitrant. In addition, decreasing nutrient loads in the bay have caused decreasing primary production (Yamaguchi and Shibata, 1979; Yamaguchi et al., 1991; Bouman et al., 2010). Therefore, DOC produced by phytoplankton should also have decreased.

4. Summary

Rapid degradation of the labile pool was observed at freshwater sites and Tokyo Bay within the first 20 days of incubation. BDOC are remineralized during the residence time of the bay water. The contribution of RDOC to the total DOC was higher than that of BDOC at all stations for the entire observation period, and accounted for 77% of the total. Accordingly, Tokyo Bay exported mostly terrestrial RDOC to the open ocean owing to the high concentration of terrestrial RDOC and faster half-lives of BDOC relative to the residence time of the bay water. The concentrations of RDOC and BDOC have decreased in the last 40 years at freshwater sites and Tokyo Bay, during which time DOC becomes more recalcitrant because of improved sewage treatment. Since organic carbon degradation occurs at STPs before being discharged, DOC flowing into the bay has decreased, especially the BDOC fraction.

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References

Aitkenhead, J. A., McDowell, W. H., and Neff, J. C.: Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters, In “Aquatic Ecosystems: Interactivity of Dissolved Organic Matter” (Findlay, S. E. G., and Sinsabaugh, R. L., Eds.), pp. 25-70, Academic Press, San Diego, 2003.

Amon, R. M. W., and Benner, R.: Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system, *Geochim. Cosmochim. Ac.*, 60, 1783-1792, 1996.

Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., Aalto, R. E., and Yoo, K.: Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere, *Front. Ecol. Environ.*, 9, 53-60, 2011.

Avery Jr., G. B., Willey, J. D., Kieber, R. J., Shank, G. C., and Whitehead, R. F.: Flux and bioavailability of Cape Fear River and rainwater dissolved organic carbon to Long Bay, southeastern United States, *Global Biogeochem. Cy.*, 17, 1042, doi:10.1029/2002GB001964, 2003.

Bauer, J. E., and Bianchi, T. S.: Dissolved organic carbon cycling and transformation, In “Treatise on estuarine and coastal science” (Wolanski, E., and McLusky, D. S., Eds.), vol. 5, pp. 7-67, Academic Press, San Diego, 2011.

Bauer, J. E., Druffel, E. R. M., Wolgast, D. M., and Griffin, S.: Temporal and regional variability in sources and cycling of DOC and POC in the northwest Atlantic continental shelf and slope, *Deep-Sea Res. Pt II*, 49, 4387-4419, 2002.

487 Bouman, H. A., Nakane, T., Oka, K., Nakata, K., Kurita, K., Sathyendranath, S., and
 488 Platt, T.: Environmental controls on phytoplankton production in coastal
 489 ecosystems: A case study from Tokyo Bay, *Estuar. Coast. Shelf S.*, 87, 63-72, 2010.
 490 Bureau of Sewerage: Management plan 2013, Bureau of Sewerage, Tokyo Metropolitan
 491 Government, pp. 117, 2013 (in Japanese).
 492 Carlson, C. A.: Production and removal processes, In “Biogeochemistry of Marine
 493 Dissolved Organic Matter” (Hansell, D. A., and Carlson, C. A., Eds.), pp. 91-151,
 494 Academic Press, San Diego, 2002.
 495 Cauwet, G.: DOM in the coastal zone, In “Biogeochemistry of Marine Dissolved
 496 Organic Matter” (Hansell, D. A., and Carlson, C. A., Eds.), pp. 579-609, Academic
 497 Press, San Diego, 2002.
 498 Chin, W. C., Orellana, M. V., and Verdugo, P.: Spontaneous assembly of marine
 499 dissolved organic matter into polymer gels, *Nature*, 391, 568-572, 1998.
 500 Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G.,
 501 Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J.:
 502 Plumbing the global carbon cycle: integrating inland waters into the terrestrial
 503 carbon budget, *Ecosystems*, 10, 171-184, 2007.
 504 Dai, M., Yin, Z., Meng, F., Liu, Q., and Cai, W. J.: Spatial distribution of riverine DOC
 505 inputs to the ocean: an updated global synthesis, *Curr. Opin. Environ. Sustain.*, 4,
 506 170-178, 2012.
 507 Druffel, E. R. M., Williams, P. M., Bauer, J. E., and Ertel, J. R.: Cycling of dissolved
 508 and particulate organic matter in the open ocean, *J. Geophys. Res. Oceans*, 97,
 509 15639-15659, 1992.
 510 Hansell, D. A.: Recalcitrant dissolved organic carbon fractions, *Annu. Rev. Mar. Sci.*, 5,

511 3.1-3.25, 2013.

512 Hansell, D. A., Carlson, C. A., Repeta, D. J., and Schlitzer, R.: Dissolved organic matter
 513 in the ocean: A controversy stimulates new insights, *Oceanography*, 22, 202-211,
 514 2009.

515 Hedges, J., Keil, R. G., and Benner, R.: What happens to terrestrial organic matter in
 516 the ocean?, *Org. Geochem.*, 27, 195-212, 1997.

517 Hernes, P. J., and Benner, R.: Terrigenous organic matter sources and reactivity in the
 518 North Atlantic Ocean and a comparison to the Arctic and Pacific oceans, *Mar.*
 519 *Chem.*, 100, 66-79, 2006.

520 Japan Sewage Works Association: Sewage statistics, 2010 (in Japanese).

521 Kadlec, R. H., and Wallace, S. D.: *Treatment wetlands* second edition, CRC Press, Boca
 522 Raton, FL, pp. 1000, 2008.

523 Kaushal, S. S., and Belt, K. T.: The urban watershed continuum: evolving spatial and
 524 temporal dimensions, *Urban Ecosys.*, 15, 409-435, 2012.

525 Kerner, M., Hohenberg, H., Ertl, S., Reckermann, M., and Spitzzy, A.: Self-organization
 526 of dissolved organic matter to micelle-like microparticles in river water, *Nature*,
 527 422, 150-154, 2003.

528 Kragh, T., and Søndergaard, M.: Production and decomposition of new DOC by marine
 529 plankton communities: carbohydrates, refractory components and nutrient
 530 limitation, *Biogeochemistry*, 96, 177-187, 2009.

531 Lønborg, C., and Álvarez-Salgado, X.A.: Recycling versus export of bioavailable
 532 dissolved organic matter in the coastal ocean and efficiency of the continental shelf
 533 pump, *Global Biogeochem. Cy.*, 26, GB3018, doi:10.1029/2012GB004353, 2012.

534 Lønborg, C., Davidson, K., Álvarez-Salgado, X. A., and Miller, A. E. J.: Bioavailability

535 and bacterial degradation rates of dissolved organic matter in a temperate coastal
536 area during an annual cycle, *Mar. Chem.*, 113, 219-226, 2009.

537 Ludwig, W., Probst, J. L., and Kempe, S.: Predicting the oceanic input of organic carbon
538 by continental erosion, *Global Biogeochem. Cy.*, 10, 23-41, 1996.

539 Maki, K., Kim, C., Yoshimizu, C., Tayasu, I., Miyajima, T., and Nagata, T.:
540 Autochthonous origin of semi-labile dissolved organic carbon in a large monomictic
541 lake (Lake Biwa): carbon stable isotopic evidence, *Limnology*, 11, 143-153, 2010.

542 Meybeck, M.: C, N, P and S in rivers: From sources to global inputs, In “Interactions of
543 C, N, P and S Biogeochemical Cycles and Global Change” (Wollast, R., Mackenzie,
544 F. T., Chou, L., Eds), pp. 163-193. Springer-Verlag, Berlin, 1993.

545 Meyers-Schulte, K. J., and Hedges, J. I.: Molecular evidence for a terrestrial component
546 of organic matter dissolved in ocean water, *Nature*, 321, 61-63, 1986.

547 Moran, M. A., and Zepp, R. G.: Role of photoreactions in the formation of biologically
548 labile compounds from dissolved organic matter, *Limnol. Oceanogr.*, 42, 1307-1316,
549 1997.

550 Moran, M. A., Sheldon, W. M. Jr., and Sheldon, J. E.: Biodegradation of riverine
551 dissolved organic carbon in five estuaries of the southeastern United States,
552 *Estuaries*, 22, 55-64, 1999.

553 Mulholland, P. J.: Formation of particulate organic carbon in water from a southeastern
554 swamp-stream, *Limnol. Oceanogr.*, 26, 790-795, 1981.

555 Nakamura, T., Osaka, K., Hiraga, Y., and Kazama, F.: Nitrogen and oxygen isotope
556 composition of nitrate in stream water of Fuji River basin, *J. Jpn. Assoc. Hydrol.*
557 *Sci.*, 41, 79-89, 2011 (in Japanese with English abstract).

558 National Institute for Land and Infrastructure Management: Information of port

559 environment, available at: <http://www.nilim.go.jp/> (last access: June 25, 2013).
 560 Nellemann, C., Hain, S., and Alder, J.: Rapid response assessment in dead water:
 561 Merging of climate change with pollution, over-harvest, and infestations in the
 562 world's fishing grounds, United Nations Environment Programme, pp.61, 2008.
 563 Nihei, Y., Takamura, T., and Watanabe, N.: Issues on discharge monitoring in main
 564 influence rivers into Tokyo Bay, J. Jpn. Soc. Civil Eng., 54, 1226-1230, 2007a (in
 565 Japanese with English abstract).
 566 Nihei, Y., Ehara, K., Usuda, M., Sakai, A., and Shigeta, K.: Water quality and pollutant
 567 load in the Edo River, Ara River and Tama River, J. Jpn. Soc. Civil Eng., 54, 1221-
 568 1225, 2007b (in Japanese with English abstract).
 569 Obernosterer, I, and Benner, R.: Competition between biological and photochemical
 570 processes in the mineralization of dissolved organic carbon, Limnol. Oceanogr., 49,
 571 117-124, 2004.
 572 Ogawa, H., and Ogura, N.: Source and behavior of organic carbon of seawater in Tokyo
 573 Bay, Chikyukagaku, 24, 27-41, 1990 (in Japanese with English abstract).
 574 Ogawa, H., and Ogura, N.: Comparison of two methods for measuring dissolved
 575 organic carbon in sea water, Nature, 356, 696-698, 1992.
 576 Ogura, N.: Further studies on decomposition of dissolved organic matter in coastal
 577 seawater, Mar. Biol., 31, 101-111, 1975.
 578 Okada, T., Takao, T., Nakayama, K., and Furukawa, K.: Change in freshwater discharge
 579 and residence time of seawater in Tokyo Bay, J. Jpn. Soc. Civil Eng., 63, 67-72,
 580 2007 (in Japanese with English abstract).
 581 Opsahl, S., and Benner, R.: Distribution and cycling of terrigenous dissolved organic
 582 matter in the ocean, Nature, 386, 480-482, 1997.

583 Raymond, P. A., and Bauer, J. E.: Bacterial consumption of DOC during transport
 584 through a temperate estuary, *Aquat. Microb. Ecol.*, 22, 1-12, 2000.

585 Raymond, P. A., and Bauer, J. E.: Riverine export of aged terrestrial organic matter to
 586 the North Atlantic Ocean, *Nature*, 409, 497-500, 2001.

587 Redfield, A. C., Ketchum, B. H., and Richards, F. A.: The influence of organisms on the
 588 composition of sea-water, In "The Sea" (Hill M. N., Eds.), pp. 26-77, Interscience,
 589 New York, 1963.

590 Regnier, P., Friedlingstein, P., Ciais, P., Mackenzie, F. T., Gruber, N., Janssens, I. A.,
 591 Laruelle, G. G., Lauerwald, R., Luyssaert, S., Andersson, A. J., Arndt, S., Arnosti, C.,
 592 Borges, A. V., Dale, A. W., Gallego-Sala, A., Godd  ris, Y., Goossens, N., Hartmann, J.,
 593 Heinze, C., Ilyina, T., Joos, F., LaRowe, D. E., Leifeld, J., Meysman, F. J. R.,
 594 Munhoven, G., Raymond, P. A., Spahni, R., Suntharalingam, P., and Thullner, M.:
 595 Anthropogenic perturbation of the carbon fluxes from land to ocean, *Nat. Geosci.*, 6,
 596 597-607, 2013.

597 Servais, P., Barillier, A., and Garnier, J.: Determination of the biodegradable fraction of
 598 dissolved and particulate organic carbon in waters, *Ann. Limnol.*, 31, 75-80, 1995.

599 Servais, P., Garnier, J., Demarteau, N., Brion, N., and Billen, G.: Supply of organic
 600 matter and bacteria to aquatic ecosystems through waste water effluents, *Water Res.*,
 601 33, 3521-3531, 1999.

602 Sholkovitz, E. R.: Flocculation of dissolved organic and inorganic matter during the
 603 mixing of river water and seawater, *Geochim. Cosmochim. Ac.*, 40, 831-845, 1976.

604 Suzuki, R., and Ishimaru, T.: An improved method for the determination of
 605 phytoplankton chlorophyll using N, N-Dimethylformamide, *J. Oceanogr. Soc. Japan*,
 606 46, 190-194, 1990.

607 Takada, H., Ishiwatari, R., and Ogura, N.: Distribution of linear alkylbenzenes (LABs)
 608 and linear alkylbenzene sulphonates (LAS) in Tokyo Bay sediments, *Estuar. Coast.*
 609 *Shelf S.*, 35, 141-156, 1992.

610 Tanaka, Y., Ogawa, H., and Miyajima, T.: Production and bacterial decomposition of
 611 dissolved organic matter in a fringing coral reef, *J. Oceanogr.*, 67, 427-437, 2011.

612 Tranvik, L., and Höfle, M. G.: Bacterial growth in mixed cultures on dissolved organic
 613 carbon from humic and clear waters, *Appl. Environ. Microb.*, 53, 482-488, 1987.

614 Yamaguchi, Y., and Shibata, Y.: Recent status of primary production in Tokyo Bay,
 615 *Bull. Coast. Oceanogr.*, 16, 106-111, 1979 (in Japanese).

616 Yamaguchi, Y., Satoh, H., and Aruga, Y.: Seasonal changes of organic carbon and
 617 nitrogen production by phytoplankton in the estuary of river Tamagawa, *Mar. Pollut.*
 618 *Bull.*, 23, 723-725, 1991.

619 Yamashita, Y., Kloeppel, B. D., Knoepp, J., Zausen, G. L., and Jaffé, R.: Effect of
 620 watershed history on dissolved organic matter characteristics in headwater streams,
 621 *Ecosystems*, 14, 1110-1122, 2011.

622 Yanagi, T., Saino, T., Ishimaru, T., and Uye, S.: A carbon budget in Tokyo Bay, *J.*
 623 *Ocenogr.*, 49, 249-256, 1993.

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Figure 1. Map of Tokyo Bay. Locations of sampling sites are indicated by black circles.

Figure 2. Changes in dissolved organic carbon ($\mu\text{mol L}^{-1}$) in surface water of (a) the upper Arakawa River station, (b) Shibaura STP, and (c) the lower Arakawa River station. Error bars represent the standard deviations.

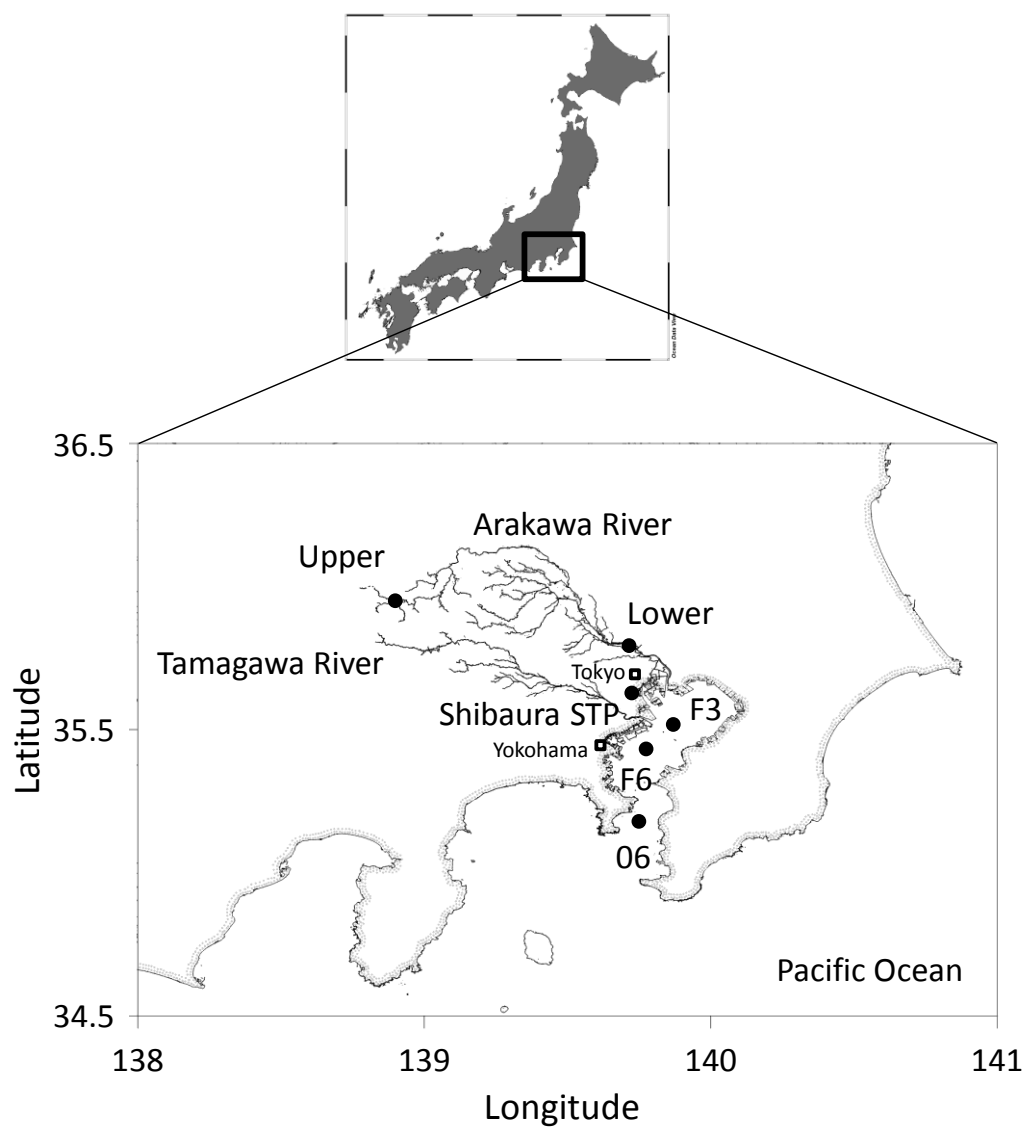
Figure 3. Seasonal variations in salinity (\square), temperature ($^{\circ}\text{C}$; \blacksquare), dissolved organic carbon ($\mu\text{mol L}^{-1}$; \blacksquare), particulate organic carbon ($\mu\text{mol L}^{-1}$; \square), and chlorophyll *a* ($\mu\text{g L}^{-1}$; \square) at station (a) F3, (b) F6, and (c) 06.

Figure 4. Changes in dissolved organic carbon ($\mu\text{mol L}^{-1}$) in surface water of (a) F3, (b) F6, and (c) 06. Black square: January 2011; gray square: February 2011; white square: March 2011; black diamond: April 2011; gray diamond: May 2011; white diamond: June 2011; black triangle: July 2011; gray triangle: August 2011; white triangle: September 2011; black circle: October 2011; gray circle: November 2011; white circle: December 2011. Error bars represent the standard deviations.

Figure 5. Seasonal variations in DOC (\blacksquare), bioavailable DOC (BDOC; \square), and recalcitrant DOC (RDOC; \blacksquare) at station (a) F3, (b) F6, and (c) 06. Error bars represent the standard deviations.

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650 Figure 1. Map of Tokyo Bay. Locations of sampling sites are indicated by black circles.

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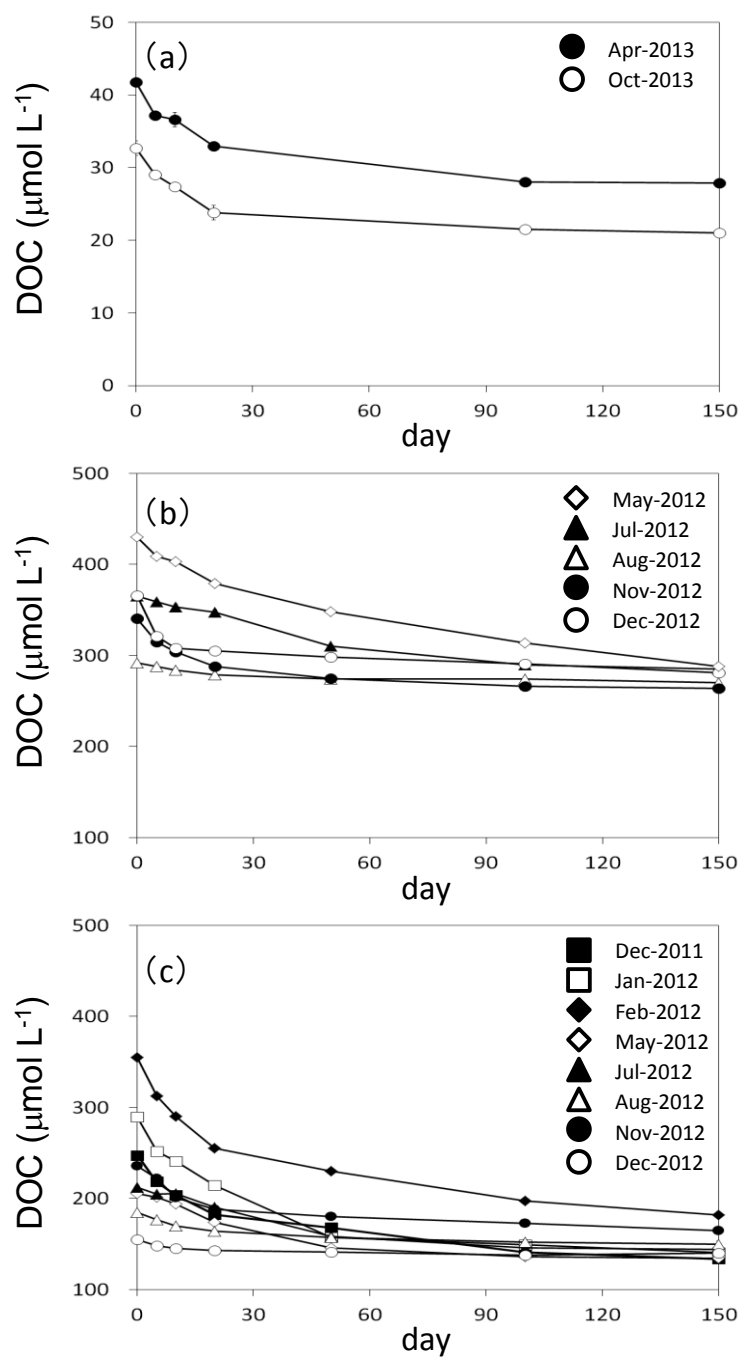
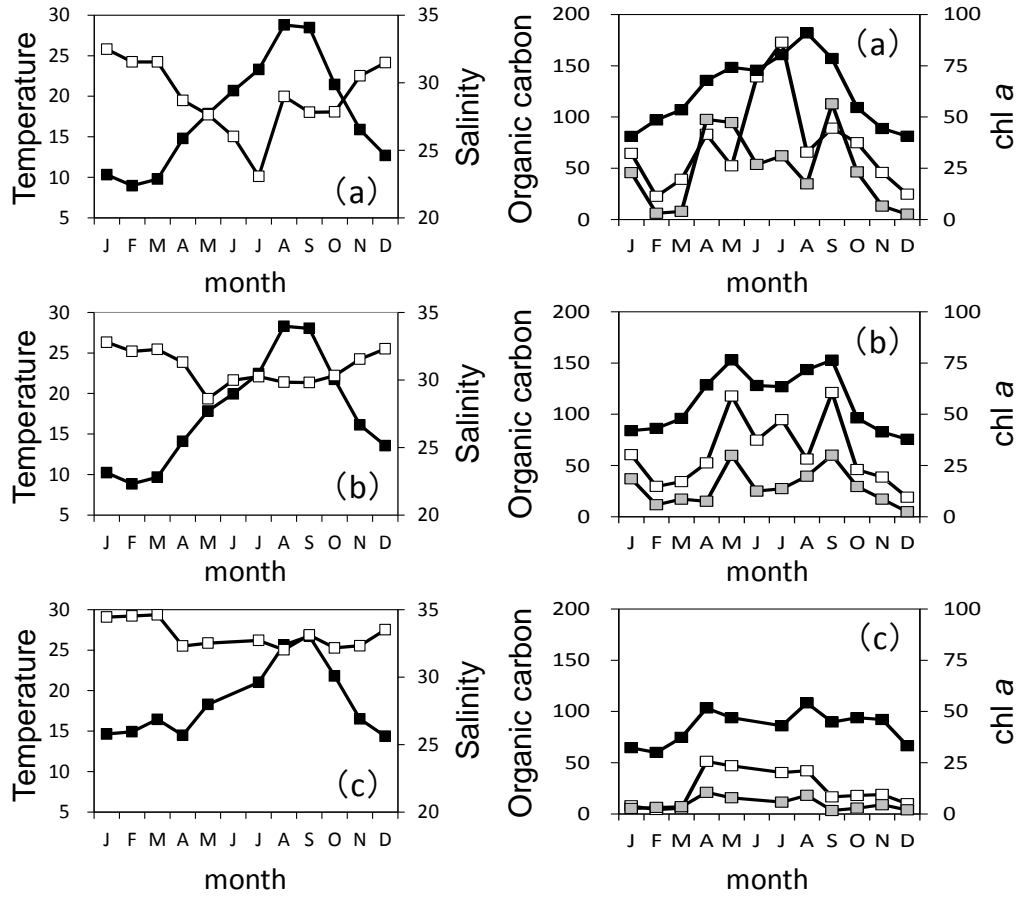


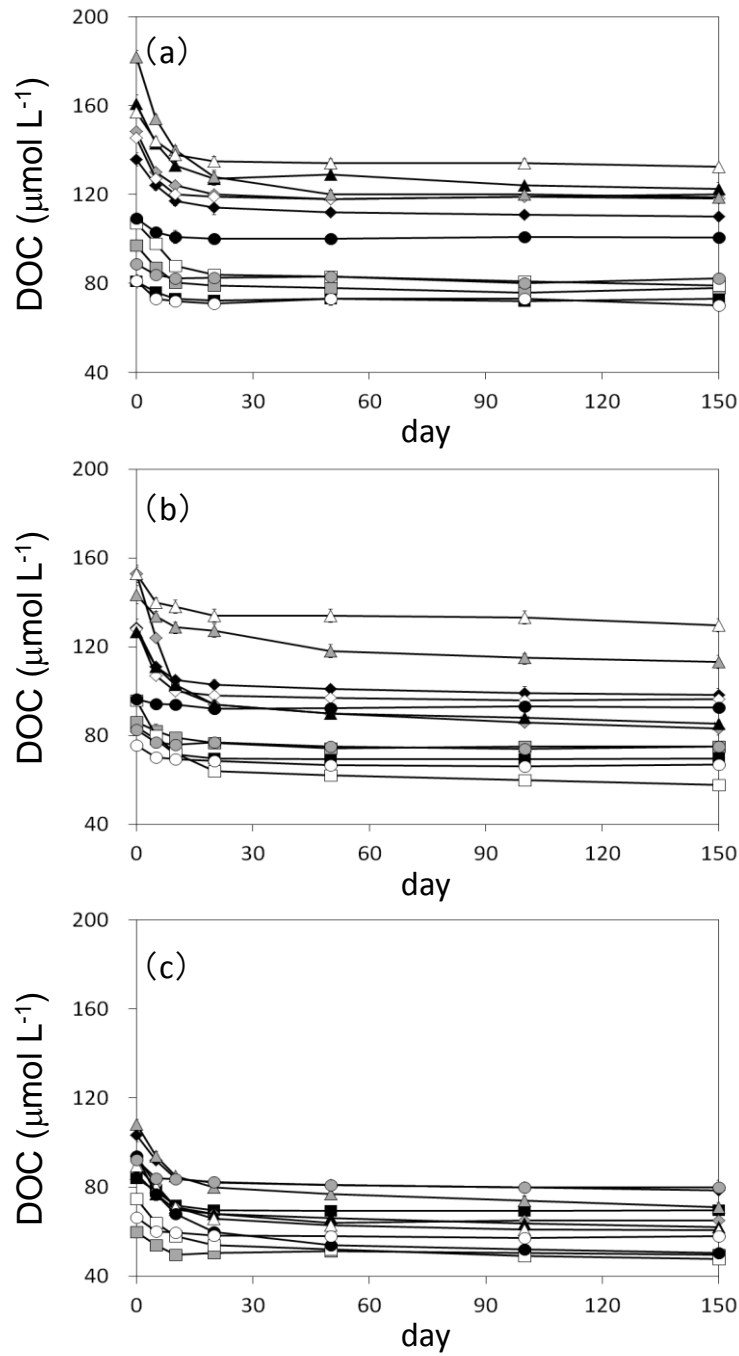
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661 Seasonal variations in salinity (□), temperature (°C; ■), dissolved organic carbon (μmol
 662 L⁻¹ ;■), particulate organic carbon (μmol L⁻¹ ; □), and chlorophyll *a* (μg L⁻¹; □) at
 663 station (a) F3, (b) F6, and (c) 06.

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667 Figure 4. Changes in dissolved organic carbon ($\mu\text{mol L}^{-1}$) in surface water of (a) F3, (b)
668 F6, and (c) O6. Black square: January 2011; gray square: February 2011; white square:
669 March 2011; black diamond: April 2011; gray diamond: May 2011; white diamond:
670 June 2011; black triangle: July 2011; gray triangle: August 2011; white triangle:

671 September 2011; black circle: October 2011; gray circle: November 2011; white circle:

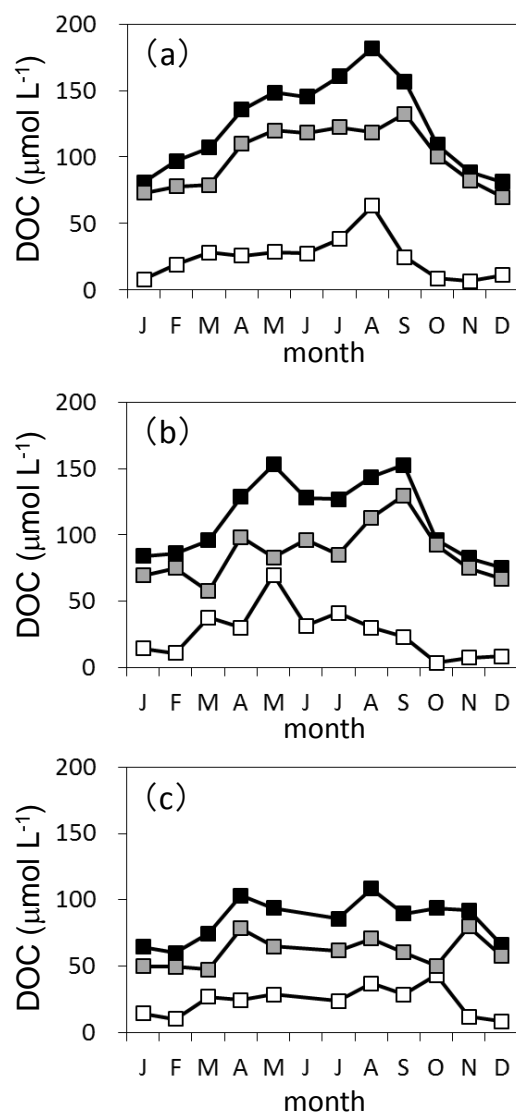
672 December 2011. Error bars represent the standard deviations.

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679 Figure 5. Seasonal variations in DOC (■), bioavailable DOC (BDOC; □), and
 680 recalcitrant DOC (RDOC; ■) at station (a) F3, (b) F6, and (c) 06. Error bars represent
 681 the standard deviations.

Table 1

Temperature (°C), salinity, chl *a* concentrations ($\mu\text{g L}^{-1}$), DOC concentrations ($\mu\text{mol L}^{-1}$) \pm standard deviation, and POC concentrations ($\mu\text{mol L}^{-1}$) at the upper Arakawa River (upper AR), the lower Arakawa River (lower AR), and Shibaura STP stations. Shibaura STP* indicates not conducted degradation experiment.

| Station | Date | Temp. | Sal. | chl <i>a</i> | DOC | POC |
|---------------|----------|-------|------|--------------|-------------|-----|
| upper AR | Apr-2013 | 10.9 | 0.0 | 0.2 | 33 \pm 0 | 13 |
| upper AR | Oct-2013 | 17.4 | 0.0 | 0.2 | 42 \pm 1 | 7 |
| lower AR | Dec-2011 | 12.1 | 0.6 | 2.0 | 247 \pm 4 | 178 |
| lower AR | Jan-2012 | 7.0 | 0.2 | 7.6 | 290 \pm 5 | 145 |
| lower AR | Feb-2012 | 7.2 | 0.2 | 49.3 | 355 \pm 3 | 313 |
| lower AR | May-2012 | 23.6 | 0.2 | 33.9 | 205 \pm 1 | 168 |
| lower AR | Jul-2012 | 24.2 | 0.2 | 1.5 | 213 \pm 2 | 84 |
| lower AR | Aug-2012 | 23.9 | 0.0 | 1.2 | 185 \pm 2 | 59 |
| lower AR | Nov-2012 | 17.4 | 0.2 | 2.0 | 236 \pm 2 | 63 |
| lower AR | Dec-2012 | 11.8 | 0.2 | 10.7 | 155 \pm 1 | 76 |
| Shibaura STP* | Jan-2012 | 14.9 | 0.4 | 0.9 | 387 \pm 2 | 191 |
| Shibaura STP* | Feb-2012 | 17.2 | 1.2 | 0.1 | 305 \pm 3 | 79 |
| Shibaura STP | May-2012 | 27.6 | 2.9 | 3.7 | 430 \pm 4 | 71 |
| Shibaura STP | Jul-2012 | 27.9 | 1.9 | 0.5 | 366 \pm 3 | 38 |
| Shibaura STP | Aug-2012 | 27.7 | 1.9 | 2.2 | 292 \pm 2 | 48 |
| Shibaura STP | Nov-2012 | 20.5 | 4.0 | 0.3 | 341 \pm 3 | 76 |
| Shibaura STP | Dec-2012 | 17.2 | 0.4 | 1.2 | 366 \pm 3 | 83 |

Table 2

Degradation constants for DOC (k_{20}) and normalized degradation constants at 15°C (k_{15}) \pm standard deviation at the upper Arakawa River (upper AR), the lower Arakawa River (lower AR), and Shibaura STP stations. R^2 indicates coefficient of determination.

| Station | Date | k_{20} (day ⁻¹) | R^2 | k_{15} (day ⁻¹) |
|--------------|----------|----------------------------------|-------|----------------------------------|
| upper AR | Apr-2013 | 0.072 \pm 0.006 | 0.99 | 0.049 \pm 0.004 |
| upper AR | Oct-2013 | 0.053 \pm 0.007 | 0.98 | 0.036 \pm 0.005 |
| lower AR | Dec-2011 | 0.038 \pm 0.004 | 0.97 | 0.025 \pm 0.003 |
| lower AR | Jan-2012 | 0.040 \pm 0.004 | 0.99 | 0.027 \pm 0.003 |
| lower AR | Feb-2012 | 0.038 \pm 0.003 | 0.96 | 0.026 \pm 0.002 |
| lower AR | May-2012 | 0.028 \pm 0.004 | 0.99 | 0.019 \pm 0.003 |
| lower AR | Jul-2012 | 0.025 \pm 0.005 | 0.99 | 0.017 \pm 0.004 |
| lower AR | Aug-2012 | 0.045 \pm 0.010 | 0.99 | 0.031 \pm 0.007 |
| lower AR | Nov-2012 | 0.052 \pm 0.005 | 0.97 | 0.035 \pm 0.004 |
| lower AR | Dec-2012 | 0.110 \pm 0.014 | 0.97 | 0.071 \pm 0.010 |
| Shibaura STP | May-2012 | 0.019 \pm 0.005 | 0.99 | 0.013 \pm 0.004 |
| Shibaura STP | Jul-2012 | 0.021 \pm 0.006 | 0.99 | 0.014 \pm 0.004 |
| Shibaura STP | Aug-2012 | 0.040 \pm 0.021 | 0.97 | 0.027 \pm 0.015 |
| Shibaura STP | Nov-2012 | 0.062 \pm 0.006 | 0.99 | 0.042 \pm 0.004 |
| Shibaura STP | Dec-2012 | 0.110 \pm 0.005 | 0.92 | 0.072 \pm 0.004 |

Table 3

Degradation constants for DOC (k_{20}) and normalized degradation constants at 15°C (k_{15}) \pm standard deviation in Tokyo Bay (F3, F6, and 06). R^2 indicates coefficient of determination.

| Station | Date | k_{20} (day ⁻¹) | R^2 | k_{15} (day ⁻¹) |
|---------|-----------|----------------------------------|-------|----------------------------------|
| F3 | Jan-2012 | 0.236 \pm 0.032 | 0.98 | 0.159 \pm 0.022 |
| F3 | Feb-2012 | 0.162 \pm 0.012 | 0.99 | 0.110 \pm 0.008 |
| F3 | Mar-2012 | 0.093 \pm 0.007 | 0.97 | 0.063 \pm 0.005 |
| F3 | Apr-2012 | 0.120 \pm 0.012 | 0.99 | 0.081 \pm 0.008 |
| F3 | May2012 | 0.203 \pm 0.009 | 0.99 | 0.137 \pm 0.006 |
| F3 | Jun-2012 | 0.286 \pm 0.007 | 0.99 | 0.193 \pm 0.005 |
| F3 | Jul-2012 | 0.127 \pm 0.010 | 0.97 | 0.086 \pm 0.007 |
| F3 | Aug-2012 | 0.109 \pm 0.005 | 0.99 | 0.074 \pm 0.004 |
| F3 | Sep-2012 | 0.153 \pm 0.010 | 0.99 | 0.103 \pm 0.007 |
| F3 | Oct-2012t | 0.301 \pm 0.025 | 0.98 | 0.203 \pm 0.017 |
| F3 | Nov-2012 | 0.269 \pm 0.024 | 0.87 | 0.182 \pm 0.017 |
| F3 | Dec-2012 | 0.221 \pm 0.019 | 0.97 | 0.149 \pm 0.014 |
| F6 | Jan-2012 | 0.150 \pm 0.016 | 0.98 | 0.101 \pm 0.011 |
| F6 | Feb-2012 | 0.095 \pm 0.020 | 0.99 | 0.064 \pm 0.014 |
| F6 | Mar-2012 | 0.100 \pm 0.003 | 0.99 | 0.067 \pm 0.002 |
| F6 | Apr-2012 | 0.151 \pm 0.011 | 0.98 | 0.102 \pm 0.008 |
| F6 | May2012 | 0.115 \pm 0.005 | 0.98 | 0.077 \pm 0.004 |
| F6 | Jun-2012 | 0.209 \pm 0.007 | 0.99 | 0.141 \pm 0.005 |
| F6 | Jul-2012 | 0.083 \pm 0.006 | 0.99 | 0.056 \pm 0.004 |
| F6 | Aug-2012 | 0.050 \pm 0.012 | 0.97 | 0.033 \pm 0.008 |
| F6 | Sep-2012 | 0.120 \pm 0.016 | 0.95 | 0.081 \pm 0.011 |
| F6 | Oct-2012t | 0.188 \pm 0.054 | 0.84 | 0.127 \pm 0.040 |
| F6 | Nov-2012 | 0.243 \pm 0.025 | 0.91 | 0.164 \pm 0.020 |
| F6 | Dec-2012 | 0.170 \pm 0.021 | 0.92 | 0.115 \pm 0.015 |
| 06 | Jan-2012 | 0.043 \pm 0.007 | 0.94 | 0.029 \pm 0.005 |
| 06 | Feb-2012 | 0.223 \pm 0.012 | 0.97 | 0.150 \pm 0.008 |
| 06 | Mar-2012 | 0.091 \pm 0.007 | 0.98 | 0.061 \pm 0.005 |
| 06 | Apr-2012 | 0.133 \pm 0.010 | 0.99 | 0.089 \pm 0.007 |
| 06 | May2012 | 0.134 \pm 0.007 | 0.99 | 0.090 \pm 0.005 |
| 06 | Jul-2012 | 0.089 \pm 0.010 | 0.97 | 0.060 \pm 0.007 |
| 06 | Aug-2012 | 0.085 \pm 0.006 | 0.97 | 0.057 \pm 0.004 |
| 06 | Sep-2012 | 0.094 \pm 0.008 | 0.99 | 0.063 \pm 0.006 |
| 06 | Oct-2012t | 0.085 \pm 0.005 | 0.99 | 0.057 \pm 0.004 |
| 06 | Nov-2012 | 0.146 \pm 0.021 | 0.94 | 0.098 \pm 0.014 |
| 06 | Dec-2012 | 0.240 \pm 0.013 | 0.87 | 0.162 \pm 0.009 |

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715 Table 4

716 Correlation coefficients (R^2) of the significant ($p < 0.05$) linear regressions between DOC

717 and hydrological data in Tokyo Bay (station F3, F6, 06, and total data). X indicates

718 dependent variable. n.s. indicates not significant.

| Station | X | Salinity | chl <i>a</i> |
|------------|------|----------|--------------|
| F3 | DOC | 0.54 | 0.36 |
| F3 | RDOC | 0.68 | 0.56 |
| F3 | BDOC | n.s. | n.s. |
| F6 | DOC | 0.74 | 0.64 |
| F6 | RDOC | 0.64 | 0.59 |
| F6 | BDOC | 0.37 | 0.31 |
| 06 | DOC | 0.81 | 0.51 |
| 06 | RDOC | 0.54 | 0.47 |
| 06 | BDOC | 0.29 | n.s. |
| Total data | DOC | 0.68 | 0.52 |
| Total data | RDOC | 0.73 | 0.62 |
| Total data | BDOC | 0.11 | n.s. |

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Table 5

Relative concentration of RDOC (%) \pm error* in Tokyo Bay derived from phytoplankton, terrestrial, and open oceanic waters estimated from two multiple linear regressions. * see the chapter 3.3.2.

| Station | Model I | | | Model II | | |
|------------|------------|------------|------------|------------|------------|------------|
| | Phyto. | Terr. | Ocean | Phyto. | Terr. | Ocean |
| F3 | 12 \pm 6 | 42 \pm 3 | 46 \pm 4 | 14 \pm 6 | 29 \pm 3 | 57 \pm 4 |
| F6 | 8 \pm 6 | 35 \pm 3 | 57 \pm 4 | 10 \pm 6 | 23 \pm 2 | 67 \pm 3 |
| 06 | 4 \pm 4 | 20 \pm 4 | 76 \pm 4 | 5 \pm 4 | 12 \pm 2 | 83 \pm 4 |
| Total data | 8 \pm 5 | 32 \pm 3 | 59 \pm 4 | 10 \pm 5 | 21 \pm 3 | 69 \pm 4 |